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Venom Alkaloids from Some *Monomorium* Species

Alexander J. Snyder, Tappey H. Jones*, Gordon C. Snelling and Roy R. Snelling

(AJS, THJ) Department of Chemistry, Virginia Military Institute, Lexington, Virginia 24450-0304, USA; email: jonesth@vmi.edu
(GCS) 13161 Rancherias Rd. Apple Valley, California 92308, USA
(RRS) Los Angeles County Museum of Natural History, 900 Exposition Boulevard, Los Angeles, California 90007, USA, deceased May 12, 2008

Abstract.—The extracts of eight species of *Monomorium* collected from 1996 to 2003 were analyzed and their characteristic venom alkaloids were identified. In each case, the peculiarity of the compounds in each species is related to previously described Myrmicine ant venoms. The taxonomic utility of these analyses is discussed.

Saturated nitrogen heterocycles have been known for over thirty years as components of the venoms of ants in the genera *Monomorium* and *Solenopsis* (Jones et al. 1982b), and these alkaloids play a well-documented defensive role in *Monomorium* species (Andersen et al. 1991). While different species of ants may have the same alkaloids, the alkaloid composition of a particular species seems to be characteristic, varying only with the age of the ants (Deslippe and Guo 2000). Comparisons of the alkaloid composition in *Solenopsis* species have been made a number of times (Brand et al. 1972; MacConnell et al. 1976; Vander Meer and Lofgren 1988). Conservatively, there are ca. 300 species of *Monomorium* worldwide (Heterick 2001), and the chemistry of a number of individual species has been reported (Jones et al. 1982b, 1989, 1990a,b, 2003; Andersen et al. 1991; Don et al. 2001). Although indolizidines, piperidines, and pyrrolizidines have been found in *Monomorium* species, 2,5-dialkylpyrrolidines are the most commonly detected alkaloids in this genus. There have been comparative studies of the alkaloids of some groups of *Monomorium* species in the United States, New Zealand and Africa (Jones et al. 1982a, 1988b, 2003), with some interest in the biological roles of these compounds; *i.e.* taxonomic value and investigation of their means to serve as defense and in predation.

There are several common structural features of the natural 2,5-disubstituted pyrrolidines found in *Monomorium* species. Most notably, the natural pyrrolidines have odd-numbered carbon skeletons and the predominance of the *trans* configuration of the ring substituents. These characteristics are easily elucidated by mass spectra in the first case and by gas chromatographic comparison with synthetic *cis/trans* mixtures in the second (Pedder et al. 1976; Jones et. al. 1979). Another important characteristic of natural pyrrolidines found in *Monomorium* species is the double bond position in the unsaturated alkyl substituents. When present, the alkyl double bonds are always terminal. Traditionally, the positions of these olefins have been verified by derivization and gas chromatography comparison with synthetic material (Jones et al. 1982b, 1988a).

In this paper, we report various alkaloids found in eight different *Monomorium* species collected in Australia, Indonesia, and Kenya from 1996 to 2003. The *Monomorium* species collected in Indonesia have yet to

* To whom correspondence should be addressed.

Table 1. Alkaloids identified from *Monomorium* species.

Species	RRS #	1	2	3	4	5	6	7	8	9	10	11
M. leae	96-449			+								
M. emersoni	01-480	4	78	17								
M. sydneyense	01-032					+						
M. rosae	01-115				+							
M. leopoldinum	03-128							1	2			
M. bifidum	03-141						2*			1	3	94*
M. species 1	98-013		1	2								
M. species 4	98-151				+							

+ = Only alkaloid detected.
* = Multiple stereoisomers detected.

be completely described, and are referred to as *M.* species 1 and *M.* species 4. In all cases except for two described species, the alkaloids are mixtures of previously reported 2,5-dialklypyrrolidines, whose structures were established by a direct comparison with synthetic samples available from previous work.

METHODS AND MATERIALS

Ants.—Collections of 10–50 workers of each species listed below were placed in a vial containing a small amount of methanol for subsequent chemical analysis. Voucher specimens of all samples are deposited in the collection of the Los Angeles County Museum of Natural History, Los Angeles, CA. RRS's collection numbers for each sample are listed in Table 1.

Monomorium leae Forel, Picadilly Circus, Brindabella Range, A.C.T., Australia;

M. emersoni Gregg, CSIRO-TERC, Berrimah, Northern Territory, Australia, 12.411°S 130.92°E, ca. 80 ft. Secondary subtropical savannah;

M. sydneyense Forel, Reef Point, Murramarang National Park; N.S.W., Australia 35.72°S 150.25°E. 0–50 m. Dry sclerophyll;

M. rosae Santschi, Laikipia Distr. Mpala Ranch, confluence of Ewaso Ng'iro and Ewaso Narok, Kenya 0.53°N 38.86°E, *Acacia* xanthophloem and *Ficus*;

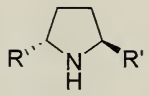
M. leopoldinum Forel, Kakamega Distr. S edge, Kalunya Glade, Kenya 0.245°N 34.870°E;

M. bifidum Heterick, CSIRO-TERC, Berrimah, Northern Territory, Australia, 12.411°S 130.92°E, ca. 25 m. Secondary subtropical savannah;

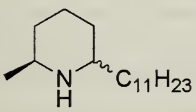
M. sp 1 and *M.* sp 4; PT. Freeport Concession, Siewa camp, Irian Jaya, Indonesia 03.04°S 136.38°E, 65 m; lowland secondary rainforest, along Wapoga River.

Chemical analysis.—Gas chromatography-mass spectrometry was carried out in the EI mode using a Shimadzu QP-5000 GC/MS equipped with a RTX-5, 30 m × .032-mm i.d. column. The instrument was programmed from 60°C to 250°C at 10°/min. Identification of the alkaloids was confirmed by direct comparison of their mass spectra and retention times with those of synthetic samples available from previous work (Fig. 1; Table 1)

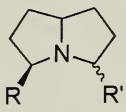
2-Butyl-5-tridecylpyrrolidine (**5**). A Stetter condensation of tetradecanal and 1-heptene-3-one (Jones et al. 1988a) provided 5,8-henecosadione in the usual manner: HRMS: Calculated for $C_{21}H_{41}O_2$ (M+1), 325.3107; observed 325.3113. Subsequent reductive amination (Jones et al 1988a) in the usual manner provided a 1:1 mixture of *cis* and *trans* 2-Butyl-5-tridecylpyrrolidine (**5**). MS *m/z* (rel%): 309(1, M^+), 308(2), 252(75), 152(3), 127(3), 126(100), 82(10, 55(12); HRMS: Calculated for $C_{21}H_{44}N$ (M+1), 310.3474; observed 310.3481. The single alkaloid detected in *M. sydneyense* had a mass spectrum and retention time identical with those of the second eluting, *trans* isomer of the synthetic mixture of **5**.



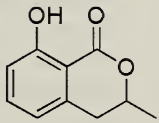
1: R = C_6H_{13}, R' = C_9H_{19}
2: R = $C_4H_8CH{=}CH_2$, R' = C_9H_{19}
3: R = $C_4H_8CH{=}CH_2$, R' = $C_7H_{14}CH{=}CH_2$
4: R = C_4H_9, R' = C_7H_{15}
5: R = C_4H_9, R' = $C_{13}H_{27}$
6: R = $C_4H_8CH{=}CH_2$, R = $C_5H_{10}CH{=}CH_2$



7: *cis*
8: *trans*



9: R = $C_2H_4CH{=}CH_2$, R' = $C_2H_4CH{=}CH_2$
10: R = C_3H_7, R' = C_5H_{11}
11: R = $C_2H_4CH{=}CH_2$, R' = $C_4H_8CH{=}CH_2$



Mellien

Fig. 1. Compounds detected in the extracts of some *Monomorium* species Australia, Indonesia, and Kenya.

M. bifidum. GC/MS analysis of the extracts of *M. bifidum* showed five nitrogen containing components in the ratios shown in Table 1. Both isomers of **6** were identified from comparison to previously published spectra (Jones et al. 1989). **9:** MS *m/z* (rel%): 219 (1,M+), 166(10), 70(9), 68(22), 67(25), 164(100), 124(10), 122(5), 41(70); **10:** MS *m/z* (rel%): 247(1,M+), 206(3), 192(80), 164(100), 124(20), 122(8), 70(10), 68(17), 67(25), 41(90); **11:** MS *m/z* (rel%): 247(1,M+), 206(1), 192(60), 164(100), 110(15), 108(4), 70(14), 68(20), 67(31), 41(92). Additionally approximately 1% of mellein was detected. Hydrogenation of a small sample of the extract over PtO_2 converted **9** to 3,5-dibutylpyrrolizidine (Garraffo et al. 1993), and **11** to the isomers of 3-butyl-5-hexylpyrrolizidine (Don and Jones 1993) which were available from previous studies.

RESULTS AND DISCUSSION

Since at least 1982, one of us (THJ) has conducted chemical analyses of ants RRS had collected. After the original chemical studies of fire ants (*Solenopsis*, subgenus *Solenopsis* spp) demonstrated differences in venom alkaloids between different species (MacConnell et al. 1976), the exocrine

chemistry of ants has been recognized as a valid taxonomic character in a number of differing groups of ants, barring some mitigating factor such as dependence on dietary sources. Often, a particular species would have some unique chemistry and RRS would then know of other related species and plan to get those on future collecting trips. In those cases we would simply wait until he had done so. The comparative study of a number of African *Monomorium* species (Jones et al. 2003) is a good example of this *modus operandi* where the collections were made over several trips. In this report we present the chemistry of the venom alkaloids of eight species of *Monomorium* from Australia, Indonesia and Kenya that were to have been markers or starting points for future sets of collections of related species, and the subsequent investigations would have most likely resulted in three separate manuscripts. The results described in this report are presented according to the structures of the venom alkaloids in the species that were examined.

The extracts of *M. leae*, *M. emersoni*,and *M.* species 1 all contained the well-known nineteen carbon 2,5-dialkyl C_6, C_9 pyrrolidines, **1**, **2**, and **3**. These compounds all have the *trans* stereochemistry regarding the attachment of the alkyl groups to the central ring, and vary only in the number of terminal carbon-carbon double bonds on their side chains. Compounds **1**, **2**, and **3** are exclusive components in the venoms of North American *Monomorium* species, in contrast with the more complex mixtures found in *Monomorium* species from New Zealand, for example. Although **1**, **2**, and **3** have also been found as concomitants with homologous bicyclic alkaloids and with alkaloids of varied carbon chain lengths in African, Australian, and New Zealand *Monomorium* species. In Australian and North American species, these compounds repel larger ants (Jones et al. 1982b; Jones et al. 1988b; Jones et al. 2003).

The extracts of *M. rosae* and *M.* species 4 contains only *trans*- 2-butyl-5-heptylpyrrolidine **4**, a previously described compound typically found in various *Solenopsis* and *Monomorium* species. Interestingly, compound **4** was actually first detected in thief ants, *Solenopsis* (*Diplorhoptrum*), as a component of their poison glands (Blum et al. 1980; Jones et al. 1982a). Compound **4** was studied more extensively after its detection in the well-known *Solenopsis fugax*, where it was shown to be a repellant of several genera of much larger ants (Blum et al. 1980). Compound **4** has also been detected as a component of a complex mixture of pyrrolidines in various *Monomorium* species, most notably *M. latinode and M. indicum*. Compound **4** was shown to be a minor component of *M. latinode's* venom (Jones et al. 1982b) and *M. indicum*, which possesses the most complex mixture of dialkylpyrrolidines ever detected in a *Monomorium* species (Jones et al. 1989). Uniquely, we were able to show that compound **4** was a single component venom alkaloid in *M. rosae* and *M.* species 4, contrasting with previously published studies involving *Monomorium* species containing compound **4**. Moreover, these *Monomorium* species were collected in disparate areas of the world (*M. rosae* – Kenya, *M.* species 4 – Indonesia), raising the question of why these two ants share the chemical similarity of having compound **4** as the sole alkaloid in their venom.

trans-2-Butyl-5-tridecylpyrrolidine **5** was detected in *Monomorium sydneyense*, a species native to the continent of Australia. This is the first report of compound **5**: a C_{21} pyrrolidine where direct comparison with synthetic material established its overall structure and *trans* stereochemistry. Although long carbon chains ($> C_{15}$) are rare in *Monomorium* species, it has been observed that other Australian *Monomorium* species contain the compound 2-ethyl-5-tridecylpyrrolodine (C_{19}) as a well-known component of their venom (Andersen et al. 1991). Interestingly, the venom alkaloids found in these Australian *Monomorium*

species resemble the 2-methylpiperidines commonly found in fire ants. A structural theme in fire ants is that the more potent venoms have longer side chains (Brand et al. 1972), which may be analogous with these Australian *Monomorium* species.

The extract of *M. leopoldinum* contained both the *cis* and *trans* isomer of 2-methyl-6-undecylpiperidine (compounds **7** and **8** respectively). Compounds **7** and **8** are 6 membered, nitrogen containing, di-substituted rings that are commonly found in thief ants, such as *S. carolinensis* (Jones et al. 1982a). Our particular findings with *M. leopoldinum* are unique because we found both the *cis* and *trans* isomers (compounds **7** and **8**) in equal amounts in a *Monomorium* species as opposed to a *Solenopsis* species. Initially, this finding led one of us (THJ) to suggest that *M. leopoldinum* was actually a *Solenopsis* species. However, RRS wittily responded in an email exchange to this attempted classification by a chemist with the following statement:

> "Well, I surely do hate to toss icy water on you all's pretty notions, but this critter is a genuine, honest to gosh *Monomorium*! In fact, nearly as I can figure, it is *M. leopoldinum* Forel. So, put that in your gas chromatograph and smoke it."

Although 2,6-dialkylpiperidines have previously been reported in the venom of *M. delagoense* (Jones et al. 1990b) this is the first report of a 2-methyl-6-alkylpiperidine, a structural type so typical of *Solenopsis* species, in a *Monomorium* species.

Of the venoms described in this paper, the venom of *M. bifidum* has proved to be the most complex. The major component (ca. 94%) of the venom of *M. bifidum* was a four to one mixture of the *exo*, *exo*-3-butenyl-5-hexenylpyrrolizidine and the *exo,endo*-3-butenyl-5-hexenylpyrrolizidine (**11**), along with ca. 5% exo,exo-3,5-dibutenylpyrrolizidine (**9**). Hydrogenation of a small sample of this extract converted **11** and **9** to the previously described 3-butyl-5-hexylpyrrolizidine (Jones et al. 1991; Don and Jones 1993) and 3,5-dibutylpyrrolizodine respectively (Garraffo et al. 1993), which were available from previous studies. In addition, trace amounts of 5-butyl-3-pentylpyrrolizidine (**10**) and 2-hexenyl-5-heptenylpyrrolidine (**6**), the monocyclic homologue of **11**, were also detected (Jones et al. 1989). The presence of **6** supports the terminal double bonds in **9** and **11**. This mono to bicyclic analogy is consistent with other *Monomorium* species, as it has been observed in *M. smithii* (Jones et al. 1990a). Additionally, small amounts of mellien were detected in the extract of *M. bifidum*. This compound is commonly found in numerous *Camponotus* species (Brand et al. 1973; Duffield et al. in Press). Mellien has never been reported from *Monomorium* or any other Myrmicine species, raising the possibility that it may be a dietary artifact in *M. bifidum*.

CONCLUSION

The genus *Monomorium* is distributed worldwide with approximately three hundred described species and a seemingly infinite number of undescribed ones. As one would expect from a genus of such taxonomic diversity, identification of the various species and forms can be extremely challenging. The results presented here and in previous papers demonstrate the potential taxonomic application of the use of venom alkaloids for identification purposes among the various *Monomorium* species. This initial chemical overview of work by RRS demonstrates the need for additional studies involving chemotaxonomic investigations of related species.

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